Coevolution in host–parasite systems

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Chapter 1

General introduction: Invasion and parasitism

It was Al-Jahiz (776-869 A.D) who first hinted that environment and migration cause changes in animal life (Shah, 2015). This means that changing environmental conditions e.g. climate change that stimulates the migration, movements of the whole populations over long distances, (Dingle and Drake, 2007; Van Buskirk *et al.*, 2009) and invasion, movements of part of populations over the long or short distances, (Ward and Masters, 2007) of organisms. Changing life from the unconsciously mobile world, e.g. plants, to the consciously mobile world, e.g. animals, has been emphasized by scholars e.g. Nasir Aldin Tusi (1201-1274 A.D) (Alakbarli, 2001). He believed that individuals could adapt to specific environmental conditions through changes of **structure** and **behaviour** (Alakbarli, 2001). However, the role of parasites, in changing the physiology and behaviour of their hosts, during the evolution of the accidental movement and deliberate movement has been ignored. The interesting question about parasites in biological philosophy is whether they have deliberate movement and manipulate the host's movement or whether they are submissive to their host's movements and do not manipulate movement of their host. Parasites could migrate with their host and could select native or invasive species as new hosts, in new habitats. The fundamental question is whether parasite coevolved with new invasive host species in the same way as previous native hosts? Any influence of the parasite on its intermediate host phenotypic manipulation in order to increase trophic transmission to the definitive host is thus thought to be favoured by natural selection. This "adaptive manipulaton" hypothesis is directly related to the concept of the "extended phenotype", introduced by Richard Dawkins (1982). As emphasized at several occassions, natural selection in a host-parasite system may not necessarily target host traits directly, but instead on the ability of parasites to alter hosts traits in a manner enhancing the trophic transmission of the parasite (Seppälä and Jokela, 2008; Seppälä *et al.*, 2008; Cézilly and Perrot-Minnot, 2010).

More than 40% of known species are parasitic (Dobson *et al.*, 2008). Parasites are a reflection of the local food-web structure and biodiversity in terms of the distributions of various hosts e.g. invertebrate groups, piscivorous fish, and waterfowl (Marcogliese *et al.*, 2006). Fitness (e.g. growth, reproduction, defense, immune competence) of individuals of native host species is related to the direct or indirect effects of parasites, e.g. *Pomphorhynchus laevis* persuades lower immuno-competence and imposes energetic costs only in its native amphipod host *Gammarus pulex*; but not in invasive host *Gammarus roeseli* (Rigaud and Moret, 2003). However, the question is whether fitness is influenced by parasite in the same way in invasive host species as in native host species? It is important to clarify the relationship between invasive hosts and the structure and behaviour of their parasites. Invasive species as a host, often show lower parasite diversity (Keane and Crawley, 2002; Torchin *et al.*, 2003) and higher parasite prevalence (MacNeil *et al.*, 2003b). According to the "enemy release" hypothesis, which states that invasive species should experience a decrease in regulation by natural enemies e.g. escape from natural predators (Keane and Crawley, 2002) and escape from natural parasitism (Dick *et al.*, 2010) to be successful in colonizing new areas.

Invasive species may introduce parasites to a new native host in a colonized area, and environmental conditions, e.g. climate change, promote future adaptations of the parasite to novel host species (Bacela-Spychalska *et al.*, 2012) or may threat native species through being a competent host for a native parasite ("parasite spillback" recommended by Kelly *et al.* (2009)). Parasite spillback impacts on invasive species may increase the infection load of native parasites in native hosts and regulate native host populations through multiple-host shared-parasite systems (Kelly *et al.*, 2009). The viability of invasive and native hosts (i.e. their survivorship, fecundity, dispersal ability, or geographic distribution) is regulated host switching mechanisms by parasite (either from the invasive host to native host or from native

host to the invasive host) (Pizzatto *et al.*, 2012). However, with both increasing geographic distance and environmental dissimilarity between localities, decreasing of similar parasite community composition in the same host species are expected (Poulin *et al.*, 2011). Maladaptation in a new host species through different migration rates between parasite and hosts (Moret *et al.*, 2007) or parasite's cryptic diversity in one location (Zittel *et al.*, 2018) are consequences of parasites host switching mechanisms.

In this chapter, I will discuss positive and negative effect of invasive species. I will show responses of native species to invasive host-species, and the effects of parasites on native and invasive host-fitness. I will show competition between native and invasive hosts could be controlled by parasites/predators. Studies on invasive hosts and parasitism are necessary for a better understanding of the behaviour of parasites during host-parasite co-evolution (Janzen and May, 1979). Host-parasite coevolution refers to hosts' ability to resist parasites under selective pressure, and parasites' ability to overcome host defenses (Mostowy and Engelsta, 2011). Mutual selection triggers coevolution between parasites and hosts (Merlo *et al.*, 2016).

1-Invasion

One of the most important ecological-global topics during the last decade is the consequences of invasion of non-indigenous species (Ruiz *et al.*, 1997). Non-indigenous species, i.e. species introduced outside their native habitat by human activity (Kolar and Lodge, 2001) or migratory hosts (Bradley and Altizer, 2005). Invasive species are non-indigenous species that spread from the point of introduction and become abundant (Kolar and Lodge, 2001). Among others, ballast water from ships that contains species collected in other areas and then dropped in new areas (Ba *et al.*, 2010), canal river connections (Van der Velde *et al.*, 1998), escaped fish from aquaculture farms (Peterson *et al.*, 2005), ornamental fish trade (Rixon *et al.*, 2005) are considered to play a major role in invasion of new aquatic species in to aquatic ecosystems.

Invasion can affect the energy flow in pelagic-benthic pathways (Macisaac, 1996). Successful invasion means that invasive species were able to expand their population more quickly than native species by taking advantage of available resources and outcompeting local populations, e.g. through decreased competition from native species or increased resources thorough eutrophication from nutrient discharge (Davis *et al.*, 2000; Wikström and Hillebrand, 2012). Invasive species effects on ecosystems could be positive (i.e. ecological services, water quality function) or negative (i.e. biodiversity loss, altering the structure and functioning of ecosystems, biodiversity function, social and economic problems). Furthermore, increasing temperatures that are due to global warming could facilitate the invasion and establishment of invasive species originating from warmer areas (Montserrat *et al.*, 2013).

2-Negative effects of invasive species

Consequences of species invasions for the future of native habitats are biodiversity loss, altering the structure and functioning of ecosystems, biodiversity function and social-economic problems, which I will discuss below.

2-1 Biodiversity loss

Invasive species and climate change are the most important proximate causes of biodiversity loss worldwide (Perrings, 2002). Invasions cause biodiversity loss and changes in native food

web structure by decreasing species richness and destroying species interactions (Galiana *et al.*, 2014). The occurrence of invasive species may cause extinction of native species (Clavero and Garcia-Berthou., 2005). Most native extinctions occur when the invasive species is a top predator (Galiana *et al.*, 2014). Invasive species may cause a change in the genetic composition and behavioural patterns of native populations (Blackburn *et al.*, 2014), causing extinction of parasites through changing native vector community structure (Dobson *et al.*, 2008; Telfer and Bown, 2012) or niche shifts (Mooney and Cleland, 2001) that lead to extinction of native species.

2-2 Altering the structure and functioning of ecosystems

Both native and invasive species have a large impact on community structure and ecosystem functioning (Hooper *et al.*, 2005). Invasion may cause reduction of activities of native species upon introduction to the new habitat; invasive species are often introduced at lower population densities than native species. The invasive species may not be able to compensate for the reduced activity of native species. For example, the replacement of native species of amphipods, e.g. *Gammarus fossarum* and *G. pulex*, by invasive species, e.g. *Dikerogammarus villosus* and *Gammarus tigrinus*, in a fresh water ecosystem resulted in a decrease of leaf litter recycling (Piscart *et al.*, 2011b). As a consequence of invasion, native predators in freshwater ecosystems, e.g. rainbow trout *Oncorhynchus mykiss* and brown trout *Salmo trutta*, lost body weight since they were feeding on the invasive New Zealand mud snail, *Potamopyrgus antipodarium*, in Green River, USA (Vinson and Baker, 2008).

2-3 Social and economic problems

The strong competitive abilities of invasive cause them to have uncontrolled population growth and causes environmental or economic damage (Lodge *et al.*, 2009). Ecologists, fish farmers and fisheries managers are interested in assessing population densities of the invasive species as predators of the fish they catch. Detailed and continuous data about the location, movements, actions, and numbers of invasive species are necessary (Boonman-Berson *et al.*, 2014). Invasive species have negative impacts on boating fouling (Lalaguna and Marco, 2008), reduce fisheries catchability through changes in fish community composition and biodiversity (Villamagna and Murphy, 2010) and reduce aquaculture productivity through pathogenic invasions (De Schryver and Vadstein, 2014). It is therefore important to exert more effort in controlling the negative impact of invasive species on fisheries and aquaculture industries. The invasive species' impacts in aquaculture are immediately clear, because the cultivated species are controlled populations, but the impacts in fishery are not immediately clear, because the species that are caught, and their habitat, are not totally under human control. The realization that invasion exists, and tolerance, control (i.e. cost of mechanical, chemical and biological removal or hunting) and management of invasive species (i.e. cost of labor, cost of equipment and the frequency of treatment) needs to get more attention from natural scientists, the media and stakeholders (Villamagna and Murphy, 2010). The goal of invasive species management is to minimize economic costs and ecological damage (Villamagna and Murphy, 2010). Any policy about control of invasive species needs detailed information about ecological, societal and economic damage (Boonman-Berson *et al.*, 2014). A study on the social impact of invasion, found that the media attributed more negative impacts to the invasive species than scientists did (Lavoie, 2010).

2-4 Biodiversity function: positive and negative

Hybridization between invasive species and native species, especially in small habitats like desert springs with low flow conditions and drought stressful condition, could lead to vigorous and fertile hybrids in F1 but the offspring of F1 hybrids (F2) are often weak and sterile in the wild (Rahel and Olden, 2008; Blackburn *et al.*, 2014). For example, F1 hybrids of non-native rainbow trout *O. mykiss* and native west slope cutthroat trout *Oncorhynchus clarkii lewisi* were shown to have reproductive success nearly equivalent to or potentially greater than native fish (Muhlfeld *et al.*, 2009). Biodiversity function in F1 is positive but F2 hybrids substantially reduce fitness and loose native species in next generation (Kovach *et al.*, 2015).

3-Positive effects of invasive species

In some instances, invasive species can also have desirable effects on an ecosystem. Invasive species can provide shelter and habitat for native species, e.g. shells of invasive Asian horn snail *Batillaria attramentaria* used as habitat by native species in pacific ocean (Wonham *et al.*, 2005). Invasive species can provide prey (i.e. invasive North American red swamp crayfish *Procambarus clarkii* in southwestern Spain) for threatened predator species (Tablado et al., 2010), or be a nutritional resource for them e.g. invasive green macro alga, *Codium fragile*, increase the recruitment of the mussel *Mytilus galloprovincialis* of sandy shores in the northern Adriatic Sea (Bulleri *et al.*, 2006). In some instances, invasive species may, therefore, be useful catalysts for ecosystem restoration e.g. *albizia sp.* plantations for carbon sequestration in grasslands in Southeast Asia (Ewel and Putz, 2004) or as biofilters for use in water clarification e.g. zebra mussel *Dreissena polymorpha* (Elliott *et al.*, 2008).

4-Response traits of native species to invasive host-species and effect of parasites on native and invasive host-fitness

How host-parasite coevolution due to different native and invasive hosts will act upon the diversity of functional responses traits? Will is cause either the host or parasite to evolve a faster growth rate? Will it lead to the host or parasite becoming more a generalist or specialist for certain traits (i.e. tolerance, competition and predation)?

4-1 Fast growth

Invasive species usually achieve higher densities and larger sizes compared to their original habitats (Torchin *et al.*, 2003). For example, in Australia, invasive cane toads, *Bufo marinus*, attain higher densities compared to native South American populations (Lampo and Bayliss, 1996) or invasive European green crabs, *Carcinus maenas*, are larger in body size in invaded area, e.g. USA, South Africa and Australia, than their European native populations. Invasive species has also bigger brood size compared to native species e.g. brood size was also greater in invasive *Gammarus tigrinus* than in native *Gammarus zaddachi* in the northern Baltic Sea (Sareyka *et al.*, 2011).

Invasive species are known as vectors of parasites in their new invaded ranges (Wattier *et al.*, 2007). Parasites have an effect on population growth of both native and invasive hosts. Parasites, e.g. cestode *Flamingolepis liguloides*, and waterfowl predators limit the population growth of native host-species, e.g. native brine shrimps *Artemia parthenogenetica*, in comparison to invasive host-species, *A. franciscana* in Aigues-Mortes saltern, South of France (Sánchez *et al.*, 2012). Parasite, microsporidium *Fibrillanosema crangonycis*, also increases the rates of population growth of its invasive host species, the North American amphipod *Crangonyx pseudogracilis*, in the UK through a combination of vertical transmission with host sex ratio distortion: female-biased sex ratios might lead to increased host population growth (Slothouber Galbreath *et al.*, 2004).

4-2 Tolerating a range of abiotic conditions: global warming, increase in salinity, eutrophication and land use.

The potential value of invasive species in providing ecosystem functions will increase in a scenario of climate change. The potential to tolerate a wider range of environmental conditions is considered to be a key to successful invasion (Ricciardi and Rasmussen, 1998). The extended anthropogenic activity throughout the last centuries, and the increasing ionic concentration in freshwaters caused by pollution and its consequences on survival of animals with high environmental tolerance has created numerous opportunities for invasive species to spread rapidly (Jazdzewski *et al.*, 2004). For example, under stressful conditions, such as increasing the salinity and temperature and decreased oxygen concentration, invasive species consistently showed more robustness in their normal respiratory performance than their native counterparts (Lenz *et al.*, 2011). For example, the invasive amphipod *G. tigrinus* was more resistant to hypoxia, insufficient oxygen, and survived at higher temperatures than the native *G. zaddachi* (Sareyka *et al.*, 2011).

In the course of the last decades, researches have focused on aquatic parasite ecology and invasion biology for understanding the role of parasites in the host communities structure (Hilker *et al.*, 2005; Emde *et al.*, 2012). Indeed, they have found that drivers (i.e. climate

change (Pellan *et al.*, 2016), parasitism (Hatcher *et al.*, 2006), and invasion (Iacarella *et al.*, 2017)) have significant effects on prey-predator relationships (Fig. 1.1).

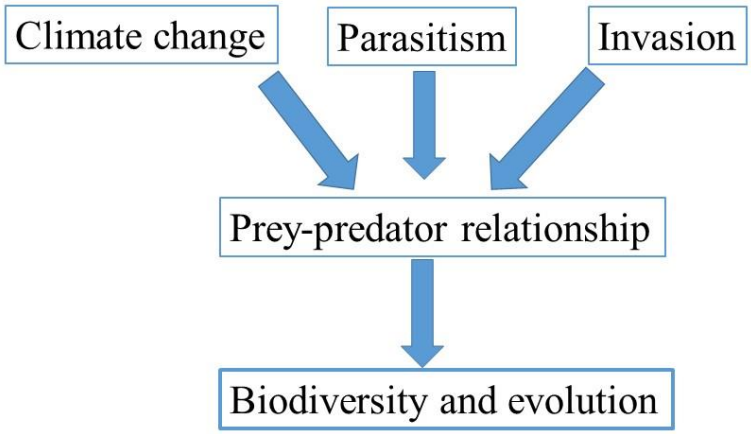

FIGURE 1.1 *Climate change, parasitism and invasion are important drivers of biodiversity and evolution.*

4-3 Competition

Competition may lead to a replacement or local extinction of one or several native species (Blackburn *et al.*, 2014). The presence of invasive amphipods, by interference competition, can lead to an increased number of native amphipods in the water column with a high accessibility to predators. For example, extinction of native amphipods was accelerated in the upper Danube River because of sympatric and synergistic effects from invasive amphipods *D. villosus*. These increased the vulnerability of native *G. pulex* to predation by invasive round goby *Neogobius melanostomus* (Beggel *et al.*, 2016).

Competition between native and invasive species can be controlled by parasites/predators through a different impact than interference competition on host fitness e.g. castration or increased predation (Prenter *et al.*, 2004; Sánchez *et al.*, 2012). Furthermore, researchers showed that the malaria parasite *Plasmodium azurophilum* reduced the competitive superiority of the native lizard *Anolis gingivinus* over the invasive lizard, *A. wattsi* and decrease blood hemoglobin (Schall, 1992). Parasites can form biological communities directly through population regulation of hosts (MacNeil *et al.*, 2003b) and indirectly by apparent competition, e.g. high attack rate by the parasite *Anagrus eposnative* in the native congeneric grape leafhopper *Erythroneura elegantula* compared to invasive variegated leafhopper *E. variabilis* alters competitive balance from equals to disadvantagous for the native population in California's San Joaquin Valley (Settle and Wilson, 1990).

4-4 Predation

Predators directly (e.g. consumption) or indirectly (e.g. strong impacts on habitat choice and feeding activity of prey amphipod *Echinogammarus marinus* (Beermann *et al.*, 2018)) have an effect on the replacement or local extinction of native species (Blackburn *et al.*, 2014). The exploitation of attractive chemical signals, such as kairomones, by predators and parasitoids are an important mechanism to locate of prey and hosts (Zhang and Schlyter, 2010). But on another level, when the preys' visual signals are limited by turbidity and darkness in an aquatic ecosystem, prey respond to chemical cues released by different predators (e.g. adjusted drifting behaviour in *G. pulex* (Dahl *et al.*, 1998)) or by activity associated with predation (e.g. injury-released chemical cues from conspecific (Wisenden *et al.*, 2001)),. However, reduced prey foraging activity and increased level of prey avoidance towards the chemical cues released by a predator reduce the predation risk (Thünken *et al.*, 2010).

4-5-1 Prey activity

Prey activity and responding to cues from predators diet seems to be a critical factor in facilitating certain behavioural responses depending on the length of co-existence with starved and conspecific-fed predators e.g. decreasing native anuran tadpoles activity in presence of native predator dragonfly *Aeshna sp*. and predated conspecific (Nunes *et al.*, 2013).

4-5-2 Predator avoidance by prey

Predator avoidance by prey is an important topic related to invasion ecology e.g. invasive amphipods *E. ischnus* avoided a larger range of fish predators than the native *Gammarus fasciatus* in Great Lakes (Pennuto and Keppler, 2008). Biological invasion contains a perfect case for studying the evolution of anti-predator phenotypic plasticity e.g. plastic responses usually occur when native *Pelodytes punctatus* and invasive *Discoglossus pictus* anurans tadpoles face recently invasive predator species, Eastern mosquitofish *Gambusia holbrooki* and crayfish *Procambarus clarkii* (Pujol-Buxó *et al.*, 2013). Differences in antipredator behaviour of species are an important fitness component in most animals with evolutionary change in their activity level (Richardson, 2001; Nunes *et al.*, 2013).

5 Aim of the thesis

The main aim of my thesis was to study the ability of parasites to alter the native and invasive intermediate host's behaviour. The arrival of invasive host species in a native host population may promote local parasite maladaptation (Moret *et al.*, 2007). In this thesis, I investigated whether parasites have the ability to alter their intermediate host's behaviour, and whether this has evolved specifically to target sympatric invasive and native host-species or host-species in general. I focused on gammarids and their parasites. I assessed the effects of parasites on behavioural traits of intermediate hosts (i.e. avoidance from the non-host predator, salinity tolerance and rheotaxis) in native and invasive populations.

6 Study system

Test animals were gammarids, their parasites and non-host predator fish in the Paderborn plateau (East-Westphalia, Germany, Fig. 1.2), i.e. the sympatric species of *G. pulex* and *Gammarus fossarum*, native to this region, and *Echinogammarus berilloni*, representing the invasive species and *Polymorphus minutus* as a parasite and three-spined stickleback,

Gasterosteus aculeatus, as a non-host predator. We carried out our study from May to September in 2009, 2010, 2011 and 2012. Gammarids are most active during the summer months, probably because of increased sexual activity (Wallace *et al.*, 1975). For this reason, we selected the summer season for our sampling time.

The Paderborn Plateau (*Paderborner Hochfläche*) in central Germany is the largest limestone and karst landscape with an area of 360 km² covering an altitudinal range from 80 to 370 m above sea level (Fig 1.2) (Meyer *et al.*, 2004). Due to the local hydro-geological conditions, most of the streams and stream sections of the Paderborn Plateau show a temporary discharge regime (Meyer *et al.*, 2004). The Paderborn plateau has an area of drying period during summertime (Meyer *et al.*, 2004). In the middle part of rivers, Alme and Altenau, are also temporary discharge areas whilst the downstream sections of these two rivers are permanent discharge areas (Meyer *et al.*, 2004).

We collected our samples from Alme, Lippe and Altenau rivers. We found the parasite *P. minutus* in some locations (see Fig. 1.3). We distinguished between infected and uninfected gammarids on the basis of orange-red spots present on the cuticle (Dezfuli and Giari, 1999). We transferred gammarids into a climate chamber in Dewar flasks, filled with water from the sampling sites. This was to keep the temperature constant and to minimize stress released during transport time (1.30 hours). The acclimation took place in a climate room at 16±1°C water temperature and dark: light cycle was 10:14 hours to simulate natural, seasonal conditions.

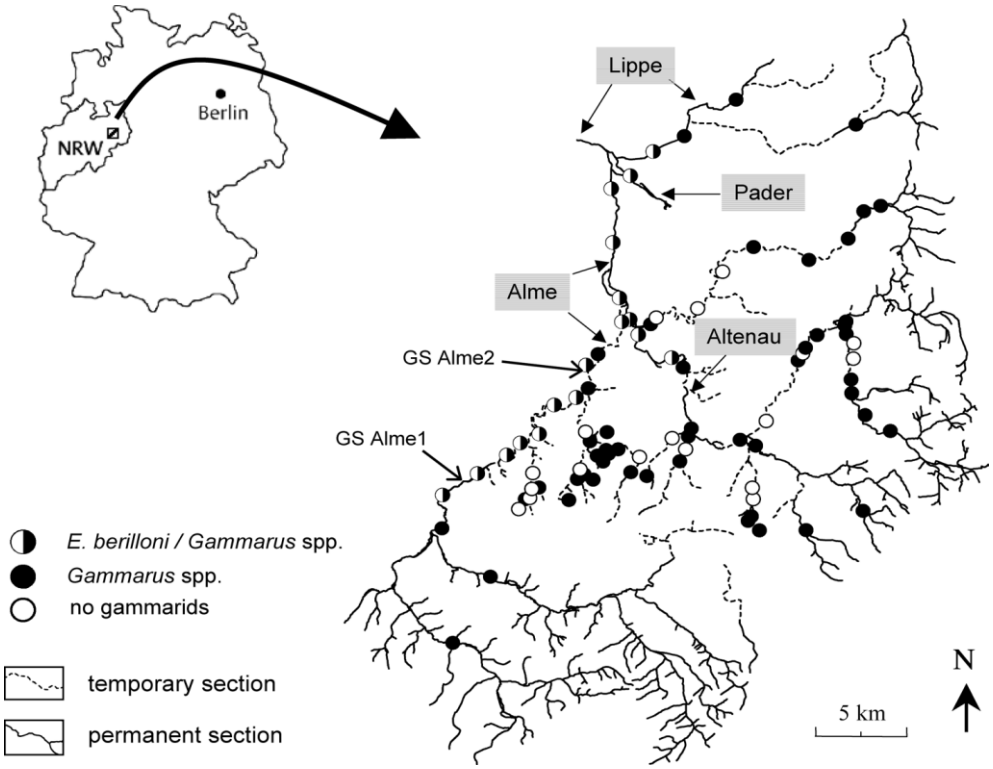

FIGURE 1.2 *The temporary karstic stream system of the Paderborn Plateau and the distribution of Amphipods recorded from 2000 to 2003: upper (Alme1) and lower (Alme2) gauging stations (GS) in the river Alme (Meyer, et al., 2004).*

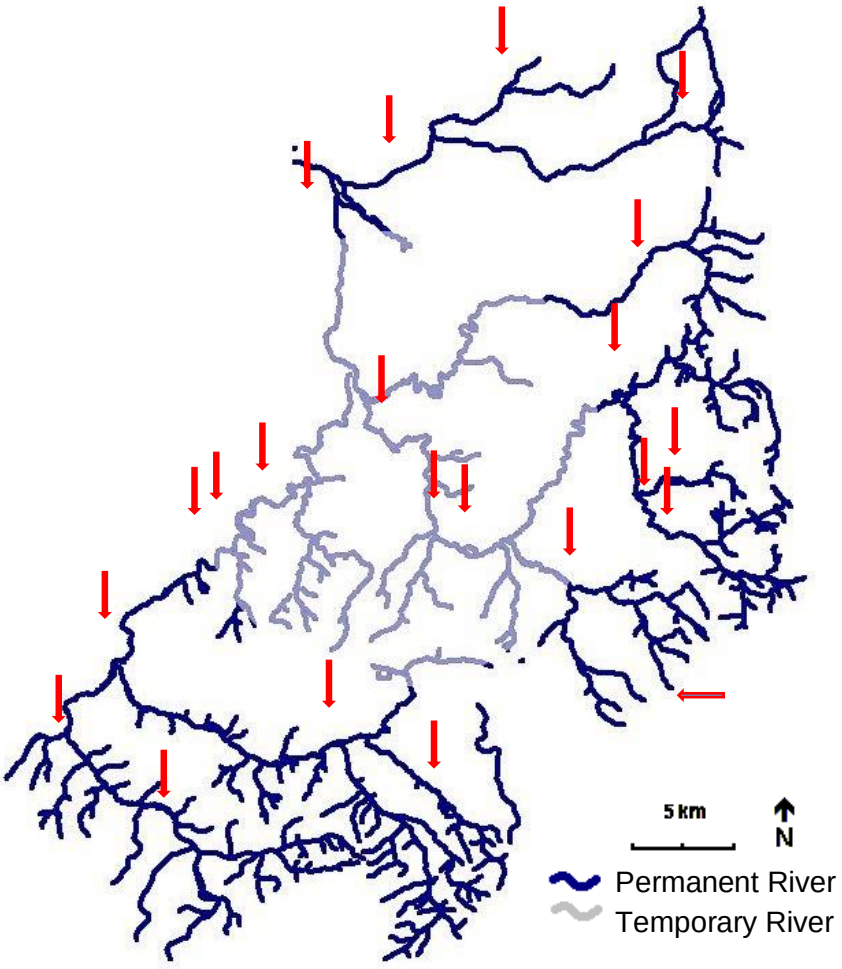

FIGURE 1.3 *Distribution of P. minutus in the Paderborn Plateau (2009-2012). Red arrows indicate locations where we have found the parasite P. minutus of gammarids during the sampling period.*

7-Acanthocephalans as an important parasite in evolutionary ecology studies

Acanthocephalans are parasites with complex life cycles involving amphipod intermediate hosts and vertebrate definitive hosts. *G. pulex* (Lingard and Crompton, 1972), *G. roeseli* (Bauer *et al.*, 2005), *G. fossarum* (Van Maren, 1979), *G. duebeni* (Hynes and Nicholas, 1963), *G. lacustris* (Hynes and Nicholas, 1963), *G. zaddachi* (Itämies *et al*,. 1980), *G. oceanicus* (Itämies *et al.*, 1980) are intermediate hosts for the acanthocephalan *P. minutus*. Acanthocephalans, with higher adaptation potential, are considered to exhibit specificity towards both intermediate and definitive hosts and it is argued that some species have switched from their intermediate host over evolutionary and historic time e.g. parasite *Echinorhynchus truttae* have used *G. duebeni* as an intermediate hosts initially but has switched to *G. pulex* in Great Britain (Lyndon and Kennedy, 2001).

The presence of *P. minutus* in gammarids is identified by an orange-red dot visible through the translucent cuticle of the infected gammarids (Fig 1.4) (Cezilly *et al.*, 2000a). *P. minutus* is able to change the geotactic behaviour of infected amphipods e.g. preferentially locate gammarids at the air-water interface and top of the water column while uninfected gammarids tend to stay at the bottom of the water column (Cezilly *et al.*, 2000a; Bauer *et al.*, 2005; Médoc *et al.*, 2009).

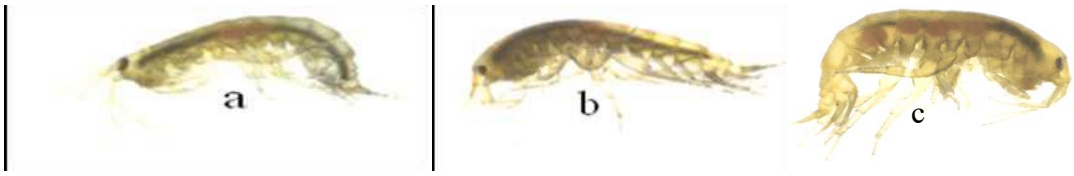

FIGURE 1.4 *Parasitized amphipods with P. minutus: a) Echinogammarus berilloni b) Gammarus pulex c) Gammarus fossarum. Photos: Markus Schmidt and Sajad Ashghali Farahani*

The mature phases of *P. minutus* occur in waterfowl that are the definitive host of this parasite, examples being: the domestic duck (Hynes and Nicholas, 1963), mallard, *Anas platyrhynchos*, tufted duck, *Authya fuligula* (Crompton and Harrison, 1965). *P. minutus* has been detected in many parts of the world (e.g. United Kingdom (Hynes and Nicholas, 1963), Germany (Zittel *et al.*, 2018), Italy (Dezfuli and Giari, 1999), USA (Canaris *et al.*, 1981), France (Cezilly *et al.*, 2000a) and Iraq (Mhaisen 1994)). During the mature phases, *P. minutus* occupied 65 to 85% of the length of the intestine in mallards, one of its definitive host (Lingard and Crompton, 1972).

Amphipods are intermediate hosts of *P. minutus*, and recognized as important components of freshwater ecosystems (Neuparth *et al.*, 2002). In Europe, populations of native amphipods have been progressively displaced by highly adaptive invasive species (Emde *et al.*, 2012). Zittel *et al.* (2018) showed that native and invasive gammarids in Westphalia, Germany (Lippe, Emscher, Ruhr and Rhine rivers) served as hosts to a cryptic *P. minutus* species. We studied three amphipods species; two native and one invasive. Both *G. pulex* and *G. fossarum* are native intermediate hosts and *E. berilloni* is an invasive, intermediate host of *P. minutus* (Fig. 1.4).

G. pulex found in small streams and abundant in medium size streams (Siegismund and Müller, 1991) in intermediate water temperatures with a possibility of adjustment to “extreme" water temperatures (5-10, 20-27°C) (Maazouzi *et al.*, 2011). *G. fossarum* is abundant in the upstream reaches of streams (Scheepmaker and Van Dalfsen, 1989; Siegismund and Müller, 1991; Müller, 1998, 2000). *G. fossarum* is adapted to intermediate water temperatures during the summer months, and needs sufficient supply of oxygen (Meijering, 1991). *E. berilloni* have been found in both a temporary and permanent karstic stream of Westphalia in Germany (Meyer *et al.*, 2004). *E. berilloni* has an endemic distribution in channels and lakes of France and Spain (Meyer *et al.*, 2004), especially in middle and lower courses of larger streams and rivers (Meyer *et al.*, 2004). There is no reliable data about *E. berilloni* temperature preferences.

8-Non-host predator

It is favorable if the intermediate host infected with *P. minutus* is preyed upon by the definitive host predators, e.g. waterfowl, because this means the parasite can complete its’ life cycle. However, if the intermediate infected host is preyed upon by a non-host species, the parasite cannot complete its life. Another possibility is that an infected intermediate host is not eaten by any kind of predator. This also has a negative effect on the parasites’ life cycle (Fig. 1.5). It is estimated that only 2.5% of the parasite population is successfully transmitted to the definitive host whereas 17.1% is predated by non-host species (Mouritsen and Poulin, 2003). The remaining (~80%) infected intermediate hosts are not eaten by any predators.

Gammarids are predated upon by non-host predators, such as three-spined sticklebacks, *Gasterosteus aculeatus* (Médoc *et al.*, 2009). Three-spined sticklebacks mainly feed on benthic invertebrates such as gammarid amphipods and isopods (Delbeek and Williams, 1988; MacNeil *et al.*, 1999).

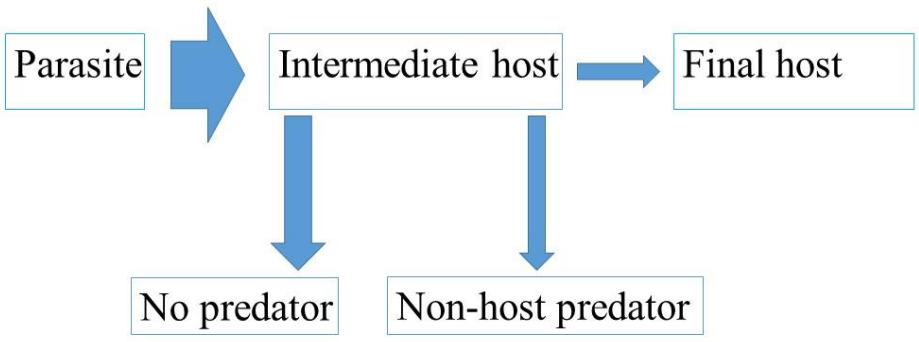

FIG 1.5 Final destination of P. minutus

9- Increased host abilities' hypothesis

The "increased host abilities' hypothesis" posits that parasites manipulate the behaviour of their intermediate hosts or improves its chances of intermediate host survival in order to enhance their transmission to the next host e.g. strong anti-predator response and highest escape speed in infected *G. roeseli* by *P. minutus* towards non-host crustacean predator *D. villosus* in order prevent inappropriate transmission (Medoc and Beisel, 2008; Beisel and Médoc, 2010). In this Hypothesis, both the parasite and its intermediate host benefit from increased host ability to survive (Medoc and Beisel, 2008). This hypothesis is in opposition of the "handicapped host' hypothesis" which emphasizes the handicapping effects of parasite on infected gammrids. For instance, the parasite *P. laevis* reduces the growth rate and oxygen consumption in its intermediate host, *G. pulex* (Rumpus and Kennedy, 1974), thereby the handicapped host becomes more conspicuous to non-host predators.

According to the "increased host abilities' hypothesis", it is the capability of the parasite to affect the host's phenotype which is the target of natural selection (Thomas *et al.*, 2005). The multi-dimensionality in parasite-induced changes of the hosts' phenotype has attracted the interest of parasitologists (Cézilly and Perrot-Minnot, 2010). Multi-dimensional host manipulation denotes the phenomenon when a single parasite alters multiple phenotypic traits of its intermediate host (Cézilly and Perrot-Minnot, 2010). Changes in the micro-distribution of intermediate hosts are often viewed as a consequence of multi-dimensional host manipulation by the parasite to increase its probability of trophic transmission (Lagrue *et al.*, 2013). Multi-dimensional manipulation does not have to be specific to be adaptive e.g., carotenoid-based colouration of acanthocephalans has no adaptive value in terms of transmission (Jacquin *et al.*, 2014). Also, when predation risk by non host predator is low, even highly nonspecific manipulation strategies can be adaptive. However, when initial predation risk is high, manipulation needs to be specific to increase parasite transmission success. (Seppälä and Jokela, 2008).

10- Competitive exclusion hypothesis

The "competitive exclusion hypothesis" predicts reducing local diversity during biological invasion by displacing native species (Muthukrishnan *et al.*, 2018). This hypothesis has an important role in describing the population regulation mechanism in the native and invasive gammarids during the presence or absence of a fish predator. For example, the invasive *D. villosus* was the stronger competitor for shelter sites compared with native *G. roeseli*, which eventually resulted in an increased mortality of *G. roeseli* in the presence of a predatory fish (De Gelder *et al.*, 2016). Evidence supporting the competitive exclusion hypothesis, where invasive species were able to outcompete native species for resources, driving them to elimination, were found by Pavlik (1983) who showed the biomass of invasive European dune grasses *Ammophila arenaria* was higher than that of native *Elyrnus mollis* in the Pacific coast of North America (Pavlik, 1983), and the invasive cordgrass *Spartina alterniflora* has larger biomass than native *S. foliosa* in San Francisco Bay (Callaway and Josselyn, 1992). Rapid growth, fecundity and a large size of invasive species increase competitive ability and contribute to invasion success (Van Kleunen *et al.*, 2010). The fate of invasive species is, in part, governed by competition between native and invasive species according to the "evolution of increased competitive ability" hypothesis (proposed by Blossey and Nötzold, 1995).

Some parasites promote competitive exclusion, others promote coexistence, and others have little effect (Frainer *et al.*, 2018). For example, in the UK, the invasive grey squirrels *Sciurus carolinensis* threatens the native competitor, the red squirrel *S. vulgaris*. Transmission of the *Parapoxvirus* from the grey squirrel causes lethal disease in the native species, but not in the invasive species (Strauss *et al.*, 2012). Parasites can promote coexistence by regulating their hosts' abundance during low infection prevalence (Dinoor and Eshed, 1984) or by reducing fitness differences between species that make coexistence more difficult (Mordecai, 2011). A parasites' competitive abilities can be maximized by increased ability of infected intermediate host to be out of reach of a non-host predator.

11- Outline of the thesis

The aim of the current thesis is to understand how parasites manipulate their intermediate host behaviour and change intermediate host salinity tolerance to facilitate successful trophic transmission to the definitive host. Fig 1.6 gives a concise overview of which experiments were done, and in which chapters they were used. Most studies to date have not investigated the "increased host abilities' hypothesis" in invasive hosts. There have also not been many investigations into anti-predatory behaviour in invasive species. To study the effects of parasite on behavioural change in native and invasive host population of gammarids, we performed an experimental to investigate prey avoidance behaviour of infected invasive *E. berilloni* and native *G. pulex* and *G. fossarum* (chapter 2). The objective of chapter 2 was to assess if *P. minutus* altered the infected gammarids behaviour towards non-host predators, more specifically three-spined sticklebacks, and if such a change was observed in all, or only sympatric, gammarid species. We tested whether the "increased host abilities' hypothesis" in non-host predators, was more pronounced in native, sympatric co-evolved gammarids in comparison to sympatric invasive gammarids. Therefore, the two native gammarid species should exhibit a stronger avoidance behaviour compared to invasive *E. berilloni*. We assessed the non-host predator avoidance between uninfected and infected gammarids during choice experiments in a Y-maze olfactometer, using water with and without three-spined stickleback cues as non-host predator.

Most studies to date have investigated the prevalence of gammarids and their parasites in aquatic ecosystems but not experimentally in the lab, and our knowledge about the behaviour of invasive species are lacking. To further investigate the information about the effects of the parasite on the gammarids' behaviour, we also performed a study (chapter 3) in which we investigated the effects of parasites on gammarid rheotaxis behaviour – to swim against or with the water current - in native and invasive populations. The research question in chapter 3 was how infection with parasites regulates rheotaxis in "sympatric" and "non-sympatric" gammarid species. We investigated how the rheotaxis controls the ecological distribution of natives during the invasion. We assessed rheotaxis between uninfected and infected gammarids during choice experiments in an open channel setup configured as an artificial water recycling canal.

Lastly, in chapter 4, we studied whether the parasite affected the salinity tolerance of native hosts. *P. minutus* depends on its intermediate host's ability to survive changing osmolality, raising the basic question whether *P. minutus* has evolved an ability to affect its intermediate hosts' salinity tolerance. Most studies to date have not shown the effects of parasites in salinity tolerance of two native species populations which overlap in their ecological niche.

We tested the competitive exclusion hypotheses: First, we expect less salinity tolerance in uninfected gammarids compared to gammarids infected with *P. minutus*. Second, we predict changes in salinity tolerance to be more distinct in *G. pulex* than *G. fossarum*. Due to the fact, they inhabit different parts of the river. To study the structural changes of the parasite we manipulated salt concentrations to investigate the salinity tolerance of native gammarids. We also studied the effect of acanthocephalan parasites on gammarid survival in different salt concentrations. We investigated if salinization in a temporary plateau could be an inhibitor factor for native species. We assessed survival in different salinities between uninfected and infected gammarids during experiments in plastic containers which were placed in plastic basins containing 12 liters of water with five different salinities.

In chapter 5 we will end this thesis with a general discussion on the results and synthesize the research findings presented in the previous chapters. We will emphasize the importance of parasite and invasive species in the habitat of the native population, especially during global soil salinization. Invasion and parasite interaction must be investigated very differently. It is expected that trophic interactions, predation, rheotaxis, an increasing salinity could lead to a variable response in different populations of native and invasive organisms, and parasites communities in aquatic ecosystems.

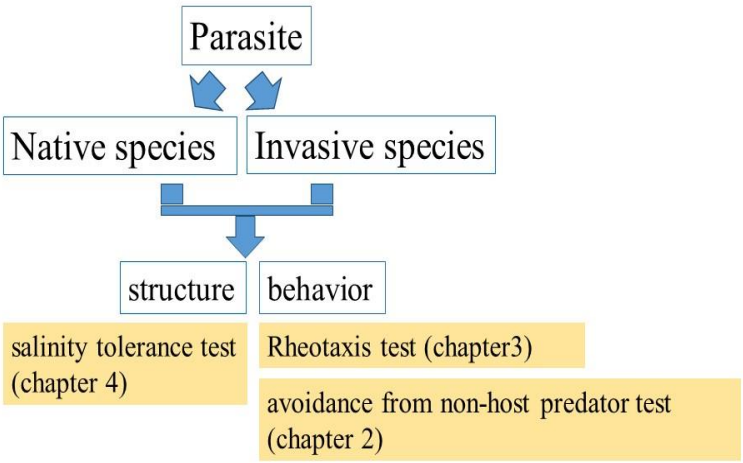

FIGURE 1.6 *Model network graphs for major incentives for interaction*

Top photo: Alme, Paderborn Plateau
Bottom photo: Altenau, Paderborn Plateau

Box 1

Effects of parasites on the behaviour of native and invasive intermediate hosts

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Amphipod communities' composition can vary substantially due to predator and parasite density (Hatcher *et al.*, 2006). Here, I discuss the mechanisms of behavioural manipulation by parasites upon their intermediate host's behaviour in relation to non-host predators and rheotaxis – to swim against or with the current.

1. Behavioural manipulation

Parasites have been shown to have the ability to alter the activity level of serotonin (5-hydroxytryptamine, 5-HT) in the brain of their intermediate hosts (Tain *et al.*, 2006a). Serotonin is a known neuromodulator of biogenic amine (Shiratori *et al.*, 2017) modulating stress response (Liang *et al.*, 2006). As a neurotransmitter, serotonin relays signals between nerve cells, or neurons (Curran and Chalasani, 2012). The serotonin contributes to a variety of physiological processes, from neuroendocrine stress response to gut contraction (Barnes and Sharp, 1999; Nichols and Nichols, 2008). Serotonin plays a role in several behavioural traits, including thermotaxic behaviour - movement of an organism in response to temperature - (Li *et al.*, 2013; Wong and Rankin, 2019), phototaxis behaviour - movement of an organism in response to light - (McPhee and Wilkens, 1989; Tain *et al.*, 2006a; Rodriguez Moncalvo and Campos, 2009; Thamm *et al.*, 2010) and geotaxis - swimming of an organism to the top or bottom of the water column - (Maximino *et al.*, 2013). The role of serotonin in feeding behaviour (Alanärä *et al.*, 1998; Ortega *et al.*, 2013) and oxygen consumption (Srinivasan *et al.*, 2008; Pérez-Campos *et al.*, 2012) have been shown by many previous researchers.

The most important role of serotonin is related to regulating escape response (Painter *et al.*, 2009) and predator avoidance (Weinberger and Klaper, 2014). Serotonin production increases with water temperature in invertebrates (Stefano et al., 1978). The metabolic rate of acanthocephalan parasites is highly dependent on temperature (Olson and Pratt, 1971; Tokeson and Holmes, 1982). The combined effects of elevated temperature and parasitic infection probably affects the performance of the gammarid brain and thus behavioural responses, such as phototaxis (Labaude *et al.*, 2017).

2. Predator cues

Most studies have shown that chemical cues from predators, in contrast with visual signals, e.g. hydrodynamic cues (i.e. incoming wave surge and water currents), remain as long as the predator is present (McIntosh *et al.*, 1999). Such cues inform prey about hunger state, densities and types of predators (Pettersson *et al.*, 2000; Brown and Magnavacca, 2003; Schoeppner and Relyea, 2005; Ferrari and Chivers, 2006; Camacho and Thacker, 2013).

The mucous of fish skin contains glycosaminoglycans (GAGs) (Van De Winkel *et al.*, 1986). The six major types of GAGs include: heparin, heparan sulphate, chondroitin, dermatan sulphate, hyaluronic acid and keratin sulphate (Rittschof and Cohen, 2004). However, only chondroitin fragments are a major component of alarm chemical cues to elicit prey behavioural response in the presence of predators (Mathuru *et al.*, 2012; Farnsley *et al.*, 2016). The chemical cues such as hypoxanthine3-N-oxide (H3NO), as an active component of the alarm pheromone system (Pfeiffer *et al.*, 1985; Brown *et al.*, 2000), and a variety of polypeptides in skin (Decho *et al.*, 1998; Wisenden *et al.*, 2009), play an important role in prey behavioural decision-making. For prey to reach maximum fitness they must have the ability to detect alarm cues of predators at an early stage in the predation sequence, before predators have detected the prey or initiated an attack (Lima and Dill, 1990; Smith, 1992). Conspecific injury cues, e.g. chemical cues released from injured prey during predatory attacks, are chemical alarms for anti- predatory responses (Wisenden *et al.*, 1999; Smith and Webster, 2015). Aggregation behaviour (Lewis *et al.*, 2012), reduced activity (Thünken *et al.*, 2010), spending more time in refuges and near the surface (Médoc *et al.*, 2009) are anti-predatory responses of gammarids after receiving chemical cues by fish or injured conspecific. Parasites alter this behaviour of gammarids, in order to enhance predation of intermediate hosts by

the definitive host predators (Jacquin *et al.*, 2014) and decrease infected intermediate host vulnerability to non-host predators (Médoc *et al.*, 2009).

3. Drifting, swimming with the current, rheotaxis

Rheotaxis is multi-sensory behaviour in which aquatic organisms tend to hold the position in the direction of flow and avoid being swept downstream by the current (Lyon, 1904; Arnold, 1974; Olive *et al.*, 2016; Oteiza *et al.*, 2017). This form of taxis is generally positive (orienting upstream and swimming against the water flow), but can also be drifting or negative (orientating downstream and swimming or being swept in the water flow) (Bureau Du Colombier *et al.*, 2009). Rheotaxis is an important behaviour for the survival of many aquatic prey species. The benefits of rheotaxis in prey, include swimming away from predator cues (Bureau Du Colombier *et al.*, 2009).

Rheotaxis is one mechanism that could facilitate the trophic transmission of parasites to their definitive hosts (Lafferty, 1999). Macneil *et al.*(2003 a,b) found parasites have the ability to alter distribution of gammarids through shifting rheotaxis. They showed a higher prevalence of parasitism in the faster and shallower water bodies (upstream) compared to slower and deeper areas (downstream).

Photo: *Gammarus pulex,* one of the intermediate hosts of the parasite thorny-headed worm *Polymorphus minutus.* Photographers: Markus Schmidt and Sajad Ashghali Farahani